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Genotypic and phenotypic diversity differences of presumptive commensal and avian pathogenic *E. coli*

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ABSTRACT

1. The objective of the experiment was to characterise the genotypic and phenotypic differences between presumptive commensal *E. coli* and avian pathogenic *E. coli* (APEC) of poultry.

2. DNA was extracted from 65 confirmed APEC *E. coli* from chicken, 100 presumptive commensal *E. coli* from healthy turkey and 35 from healthy chicken. Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and virulence factors genotyping was performed to characterise genetic features.

3. Carbon source utilisation and antimicrobial susceptibility tests were performed to characterise phenotypic features of isolates.

4. The genetic divergence between *E. coli* strains tested by ERIC-PCR profiles and virulence associated genes showed a clear genetic separation between *E. coli* APEC and turkey *E. coli* strains.

5. The carbon utilisation profile of turkey isolates was different from chicken and APEC strains; whereas antimicrobial susceptibility was highest for turkey isolates (53%), and lowest for APEC strains (33.8%).

6. The study showed a significant negative correlation between utilisation of arabitol and adonitol with different virulence determinants tested, which suggests that the ability to utilise some uncommon carbon sources may be used to discriminate between presumptive commensal *E. coli* and APEC.

Keywords: APEC; *E. coli*; commensal; virulence genes; antimicrobial resistance; carbon source.

Introduction

Escherichia coli is one of the most frequent causal agents of common bacterial gastro-intestinal infections for both human and animals (Allocati *et al.* 2013). These bacteria are classified into a number of specific pathotypes such as enterotoxigenic, enteropathogenic, enteroinvasive, or enterohaemorrhagic according to the presence of specific virulence factors (Nataro and Kaper 1998). The differentiation between non-pathogenic commensal and pathogenic types of *E. coli* that may be present in the microflora of the intestinal tract of poultry at any one time remains a topic of considerable debate. For example, having 40 virulence determinants is sufficient for an *E. coli* isolate to be described as an APEC type (Johnson *et al.* 2008), although it can be suggested that carriage of seven to nine virulence determinants may be a more accurate definition (Cordoni *et al.* 2016). Accurate differentiation is complex as it is not possible to perform infection studies in poultry for every isolate to confirm Koch's postulates. Moreover, pathogenic types residing in the gut before inducing disease, often occur as an opportunistic secondary infection to prior disease such as mycoplasmosis, which can confound results.

Traditionally, isolates belonging to O1, O2 and O78 serotypes are regarded as pathogenic (Ewers *et al.* 2003, Wang *et al.* 2014). Yet, this monothetic test is fallible because many other serotypes harbour APEC characteristics (Johnson *et al. et al.* 2008, Cordoni *et al. et al.* 2016). Several of the more commonly reported virulence factors associated with APEC include increased serum survival (*iss*), presence of type 1 fimbriae (*fimH*), P fimbriae (*papC*), and temperature-sensitive hemagglutinin (*tsh*) of the autotransporter group of proteins (Delicato *et al. et al.* 2003, Ewers *et al.* 2004). These and many other virulence factors may be used to investigate isolates to differentiate between APEC strains associated with specific syndromes (Johnson *et al. et al.* 2008) but using these to differentiate commensals from

APEC remains complicated. Whilst whole genome sequencing (Paixao *et al. et al.* 2016) is probably the most appropriate way to gain insights into these differences, it would be interesting to determine a set of relatively simple and readily transferable tests that front-line diagnostic laboratories, rather than specialist reference laboratories, can undertake. The aim of this preliminary study was to investigate the genotypic and phenotypic diversity of *E. coli* populations in poultry.

Materials and Methods

E. coli strains

Sixty-five defined and fully sequenced APEC isolated from *E. coli* infected, diseased chickens were provided by the University of Surrey (Cordoni *et al.* 2016), 35 isolates from caecal swabs from 35 healthy chickens and 100 isolates made from caecal swabs from 100 healthy turkeys reared at the University of Reading [CEDAR] farm, to give a total of 200 isolates. Chicken and turkey swab samples were grown on MacConkey agar and incubated at 37°C for 18-24 h aerobically. Well-isolated single pink colonies were picked and sub-cultured on selective media eosin methylene blue (EMB) to confirm the isolation of *E. coli* by lactose fermentation and acid production, shown as dark blue-black colonies with metallic green colonies. After isolation, *E. coli* were confirmed further using an indole test and API-20E test kit according to manufacturer's protocol (BioMerieux, Lyon, France).

Genomic DNA extraction and ERIC-PCR

DNA was extracted from the 200 isolates using QIAGEN Puregene yeast/bacteria kit B and the manufacturer's standard protocol for fresh samples of Gram-negative bacteria were followed. DNA concentration was determined with a ND-1000 spectrophotometer, and DNA stocks at 100 ng/µl were prepared and stored at -20˚C until use. *E. coli* fingerprinting was

performed using Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR). The specific primers were ERIC1: 5′-ATGTAAGCTCCTGGGGATTCAC-3′, and ERIC2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′ (Ramazanzadeh *et al.* 2013). Each ERIC-PCR reaction was carried out in a total volume of 50 µl Promega PCR mixture comprised of 100 ng of *E. coli* DNA, 1 µl (25 pmol) of each primer, 1 µl (200 mM) of dNTP mixture, 10 µl of 5X buffer solution, 4 µl (25 mM) of $MgCl_2$, and 1 µl (1.0 U) of *Taq* DNA polymerase (Promega Corporation, WI, USA). PCR amplification conditions were the same for all reactions and were carried out according to the following; initial denaturation at 94˚C for 2 min, followed by 35 cycles of denaturation at 95˚C for 30s, annealing at 52˚C for 30s, extension at 72˚C for 1 min, and a final extension step at 72˚C for 5 min. Amplicons were examined by 1.5% TAE agarose gels electrophoresis. The data was analysed visually with DNA band sizes determined using the detect band button of NTSYSpc software Version 2.1b (Exeter Software, NY, USA).

Virulence genotyping

All *E. coli* samples were analysed for eleven APEC virulence factors using PCR assays. Virulence factor selection was based on the most virulent determinants associated with different diseases in poultry. Target genes and their descriptions are summarised in Table 1 with their respective primer sequences. All PCR assays were performed as above with 25 µl of Promega PCR mixture and 1 µl (25 pmol) of each primer. PCR amplification was according to the following; initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C, for 30s, annealing at 58°C for 30s, extension at 68°C for 3 min, and the final extension step at 72°C for 5 min. Analysis of the amplified products was performed by 1.5% agarose gel electrophoresis.

Table 1 here

Carbon source utilisation test

Two hundred strains of isolated *E. coli* were tested for their ability to utilise 11 substrates as sole sources of carbon and energy. Two to three colonies of each strain were inoculated on minimal salts medium (M9) prepared according to (Lee *et al.* 2010) with modification of the following carbon sources (0.2% w/v): sucrose (suc), raffinose (raf), inositol (ino), adonitol (ado), arabitol (ara), dulcitol (dul), allantoin (all), proline (pro), sorbose (sor), melitzitose (mel), and salicin (sal). *E. coli* strains were streaked on to each plate and incubated at 37°C for 18-24 hours, then incubated at 25˚C for up to seven days. Results were recorded after 7 days' incubation.

Antimicrobial susceptibility test

Antimicrobial sensitivity was determined for the 200 isolated samples using the disc diffusion method (Bauer *et al.* 1966). Antimicrobial infused discs (Mast group, Mastdisks, UK) were used according to the standards and interpretation criteria described by National Committee for Clinical Laboratory Standards NCCLS/CLSI guidelines (Hsueh *et al.* 2010). The following antibiotics were used: nalidixic acid (10µg), amikacin (30µg), ampicillin (10µg), streptomycin (10µg), colistin sulphate (10µg), chloramphenicol (30µg), and trimethprim (5µg). *E. coli* were spread over the surface and plates were incubated at 37°C for 18-24 hours and susceptibility was determined by measuring the zone of inhibition. The diameter of the zone of growth inhibition around each disc were measured and compared with zones of inhibition of standard controls according to standards of the NCCLS/CLSI (CLSI 2018).

Data analysis

ERIC fingerprint analyses were entered in the SAHN feature of the NTSYSpc software Version 2.1b for the construction of dendrogram based on simple matching coefficient and

UPGMA (Unweighted Pair Group Method for Arithmetic Averages) in cluster analysis to determine the genetic relatedness of the *E. coli* strains (Exeter Software, NY, USA). To determine the diversity between all characteristics tested by genotypic and phenotypic tests; a multivariate analysis of Principal Components Analysis (PCA) was used. The Pearson's correlation between all characteristics tested was determined by using Minitab version 17 software (Minitab, Inc., Coventry, UK). P-value of <0.05 was taken to indicates statistical significance.

Results

Genotypic diversity

The ERIC-PCR genotyping method results show the number of bands present, different sizes, and characteristic band patterns of *E. coli* strains' DNA fingerprints. One hundred and seventy-five fingerprinting patterns were determined among the 200 isolates. The sizes of the PCR products ranged from slightly less than 150 bp to > 3000 bp with products ranging from 450-1250 bp most commonly encountered. Out of the 200 isolates strains, 175 (87.5%) were grouped in 31 groups that shared banding patterns indicative of a similar origin, and 25 (12.5%) isolates displayed a single profile. A dendrogram based on simple matching coefficient (Figure 1) showed that at a coefficient of 0.089, 31 clusters and 25 singletons at a D value of 0.848 were identified. The index indicated that, if two strains were randomly sampled from the population, then, on 84.8% of occasions, they would fall into different types. Cluster 1 was the biggest cluster in the dendrogram having 76 *E. coli* strains and most of them were isolated from turkeys and only five from chickens. Similarly, *E. coli* strains in cluster 2 to cluster 10 were related to each other more than those in clusters 11 and 12, as those strains were mixed turkey and chicken *E. coli* isolates. *E. coli* strains in clusters 16 to 22 were related to each other however, strains in clusters 17 to 22 were isolated from APEC

strains. Twenty-five singleton (single isolates) *E. coli* strains were heterogenous and showed more distant relation to other *E. coli* strains at a coefficient of 0.089, although, all singletons were isolated from chicken (12), and APEC (12) strains except for one isolated from turkey.

Figure 1 here

Two hundred *E. coli* strains were investigated for the presence of 11 virulence associated genes. The most frequently detected gene was *crl* that regulates the curli fimbrial operon, which was found in all strains (99.5%) except for one turkey isolate. Figure 2 illustrates the detection frequency of each virulence factor assessed. Using the criterion that *E. coli* should have five or more virulence associated genes to be classified as APEC, these results show that 184 strains out of 200 strains could be classified as APEC strains according to that criterion (Table 2). However, if the criterion of seven or more genes was used to classify APEC then 119 strains would be defined as APEC and less than half (37%) of the turkey isolates would be defined as APEC. Whereas, 82.8% of chicken isolates would be classified as APEC, and 81.5% of the chicken confirmed APEC isolates would be classified as APEC by the current assessment.

Table 2 here

Figure 2 here

To show the genetic divergence between *E. coli* strains generated by ERIC-PCR profiles and virulence associated genes, PCA analysis was performed. Score plots between the first two components for which the eigenvalues were most significant (6.1 and 2.6, respectively) showed the clear separation between turkey and APEC strains (Supplementary material S1). However, presumptive commensals from chickens were located between turkey and APEC strains.

Phenotypic diversity

A large subset of the 200 isolates were able to utilise the following carbon sources; dulcitol (galactitol) (38%), sucrose (37.5%), raffinose (36%) and sorbose (34.5%), irrespective of their source of isolation. Fewer strains, notably those isolated from APEC, could utilise proline (7%), inositol (3%), and salicin (1%). No strains utilised either melezitose or allantoin. Most strains that could utilise raffinose were able to utilise sucrose. Strains isolated from Turkey utilised adonitol (ribitol) and arabitol, while other strains from chicken and APEC could not. (Figure 3).

Figure 3 here

The 200 *E. coli* isolates were tested for antimicrobial susceptibility against seven antimicrobial agents using the disc diffusion method. The total number of isolates susceptible to all antibiotics tested was 87 (43.5%). Susceptibility frequencies were 53% for turkey isolates, 34.2% chicken isolates and 33.8% for APEC isolates. APEC were resistant to most antimicrobials tested compared to either from turkey or chicken isolates but resistance to

nalidixic acid and ampicillin was noted in a relatively high frequency (36%-39%) irrespective of the source of the isolates tested (Table 3).

Table 3

To estimate of the phenotypic diversity between *E. coli* isolates, a PCA was performed using carbon source utilisation and antimicrobial susceptibility variables. Score plots between the first 2 components for which the eigenvalues were 3.95 and 2.12 respectively, showed little separation between *E. coli* isolates, irrespective of origin (data not shown).

Diversity Analysis using Combined Genotypic and Phenotypic Data

To estimate diversity, the correlation between all genotypic and phenotypic characteristics in combination were evaluated. Figure 4, represents the plot of the phenotypic distance *vs*. the genotypic distance between the isolated strains for which the eigenvalue for the first two components were highly significant (8.63 and 3.06; respectively). Turkey strains as well as 26 chicken isolates were clearly distant, both genetically and phenotypically, from APEC isolates and nine chicken isolates.

Figure 4

The relative diversity of *E. coli* isolates, as assessed by both genotypic and phenotypic tests, is shown graphically in Figure 5. Some variables were co-located into distinct two groups (A and B), and these groups are more likely to share specific traits indicating a high correlation

between them. Group A showed clustering of many different antibiotic resistances, possibly because antibiotic resistance is often co-located on plasmids. Therefore, this clustering indicated acquisition of plasmids rather than any other trait. Whereas, group B were able to utilise proline, sucrose, raffinose, sorbose and dulcitol, and carried *iucD, iss, cva/cvi* and K1 virulence genes.

Figure 5

Significant correlations (P<0.05) between virulence genes and phenotypic characteristics in *E. coli* isolates were found for some variables (Supplementary S2). Arabitol and adontiol (ribitol) utilisation were negatively correlated with many virulence factors including *iucD, iss, cva/cvi* and k1 capsule (r=-0.234, -0.308, -0.201 and -0.231; respectively) and were inversely correlated with other carbon sources including; raffinose, dulcitol, proline, sorbose and sucrose. Conversely, utilisation of proline was associated with the same variables as arabitol and adonitol, but with a positive Pearson correlation with *iucD, iss, cva/cvi and k1* (r = 0.258, 0.195, 0.26 and 0.392).

Discussion

Whilst there is increasing understanding of the diversity of APEC, the aim of this experiment was to determine whether relatively simple diagnostic laboratory tests could distinguish pathogenic strains from their commensal counterparts. The investigation used *E. coli* isolated from healthy chickens and turkeys from dietary investigation studies at CEDAR, the University of Reading farm, for comparison with confirmed APEC strains from previous work.

The ERIC-PCR genotyping, in the main, grouped *E. coli* isolates by source and type confirming both the utility of this method and the findings of others (Maurer *et al.* 1998, Versalovic *et al.* 1991, Namvar and Warriner 2006).

Virulence factor genotyping of the 200 isolates revealed that a high percentage of them harbour virulence factors related to adhesion, iron acquisition and serum resistance, which are characteristic of the APEC pathotype (Delicato *et al. et al.* 2003). The most common virulence factors *fimH, crl, irp2* and *csg* were present in almost all the isolates, so these may not be associated with APEC specifically but may be considered ubiquitous systems for all *E. coli*. However, this does not preclude the fimbriae (type 1 and curli) from being considered potential virulence determinants, as they facilitate adherence which is important in the early stages of pathogenesis. The frequency of *tsh, papC, astA, iucD,* and *kps* detection differed from previous reports (Dozois *et al. et al.* 2000, Zhao *et al. et al.* 2009, Guastalli 2013) and the *iss* gene was detected in the current isolates at a higher frequency than previously seen (Delicato *et al.* 2003, Rodriguez-Siek *et al.* 2005). These discrepancies might be explained by the limited selection of isolates used and the fact that the *iss* gene is carried on a plasmid associated with chicken species. Additionally, *cva/cvi* was present in 80% of APEC isolates suggesting that *cva/cvi* could be considered a potentially defining feature of the APEC strains (Vandekerchove *et al.* 2005). Interestingly, if exclusion of three common virulence genes (*crl, fimH* and *csg*), which were found in high percentages (99.5%, 97% and 95.5%; respectively), is considered, followed by applying the classification of *E. coli* into APEC if they harbour 5 or more virulence genes; the results changed to 13% APEC classified in turkey, 62.8% APEC from healthy chicken isolates, and 53.8% in the confirmed APEC isolates from chicken. Therefore, the question remains whether the selection of virulence genes for testing using the defined ~40 genes might be beyond a diagnostic laboratory, but remains a more relatively accurate method for APEC classification. Moreover, it is possible

that other factors participate in the accurate classification of APEC strains, such as phenotypic characteristics.

The PCA plots suggested that presumptive commensal strains from chicken appear to share similarities with and sit between turkey presumptive commensals and APEC in terms of shared factors. The turkey isolates generally possessed far fewer virulence determinants whereas chicken isolates showed greater diversity, which probably represented genuine commensals. The data suggested that turkey isolates are more alike and may therefore be adapted to the turkey as a host. Given that *E. coli* related disease in turkey is relatively rare, the data suggested two possibilities. First, that these are genuine turkey related commensals lacking many virulence determinants or second, some are potential turkey pathogens but have yet undefined virulence determinants that are different from those associated with chickens.

The genotypic data suggested that the *E. coli* from healthy chickens were very diverse and many were potentially pathogenic. Interestingly, the results showed a higher than anticipated utilisation rate for L-sorbose, raffinose and dulcitol in APEC isolates. Interestingly, each of the three group of isolates could utilise, variably, only six substrates with the proportion of strains able to utilise a substrate dependent on the host from which they were isolated. This observation may indicate selection that was dependent upon what the animals were fed (Souza *et al.* 1999) and may be considered as possible discriminatory substrates for screening and identification of pathogenicity (Ratnam *et al.* 1988, Durso *et al.* 2004).

Only presumptive commensal strains isolated from turkeys utilised adonitol (ribitol) and arabitol, although these two sugars (pentitols) are relatively abundant in nature. Woodward and Charles (1983) demonstrated that the genes for dulcitol (galactitol) utilisation and those for adonitol and arabitol utilisation are mutually exclusive at minute 44 on the *E. coli* genome (Woodward and Charles 1983). It seems likely that testing for growth on these pentitols may

provide the easiest way to distinguish commensal *E. coli* strains of turkeys from chicken strains if this correlation can be confirmed by further studies that make use of a much wider collection of *E. coli* sources. The ability to utilise proline as a sole energy source was limited to APEC strains, although only 21.5% were positive for this characteristic. Whether this will be a useful differential marker requires further testing.

Antimicrobial treatment is the most commonly used control measures for reducing morbidity and mortality caused by APEC in poultry (Dho-Moulin and Fairbrother 1999, Guerra *et al.* 2003) but has the consequence of high incidence of resistance. Whilst many isolates were susceptible to most of the antimicrobials tested, confirmed APEC isolates showed the highest resistance, confirming the findings of other reports (Kazemnia *et al.* 2014, Salehi and Bonab 2006, Mellata 2013).

There was a significant negative correlation between utilisation of the carbon sources arabitol and adonitol with the different virulence determinants tested, such as, *iucD, iss, cva/cvi* and k1. Moreover, utilisation of proline was associated with the same genetic variables as arabitol and adonitol but with a positive correlation with *iucD, iss, cva/cvi and k1*. Thus, a strong correlation between the virulence genes and phenotypic characteristics suggested that the ability to utilise some uncommon carbon sources may discriminate between presumptive commensal *E. coli* and APEC. Whilst it is recognised that these hypotheses require a wider study using isolates made from many different geographic locations and management systems, if confirmed, simple tests may enable differentiation of APEC from harmless commensals. Based on these findings, the next step is confirmation of Koch's postulates in appropriate *in vivo* models.

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Table 1: The primers used for detection of the various genes by PCR, amplicon size, encoded virulence factors and primer references were used.

Gene	Description	Primer sequence	Amplicon size (bp)	References
fimH	Type 1 fimbriae adhesion	AGAACGGATAAGCCGTG GCAGTCACCTGCCCTCCGGTA	508	(Zhao *et al.* 2009)
papC	P-fimbriae, Pyelonephritis associated pili	TGATATCACGCAGTCAGTAG CCGGCCATATTCACATA	501	(Janben *et al.* 2001)
csg	Regulator of the curli fimbriae operon	ACTCTGACTTGACTATTACC AGATGCAGTCTGGTCAAC	200	(Knobl *et al.* 2012)
crl	Curli fiber gene	TTTCGATTGTCTGGCTGTATG CTTCAGATTCAGCGTCGTC	250	(Knobl *et al.* 2012)
tsh	Temperature-sensitive hemagglutinin	ACTATTCTCTGCAGGAAGT CTTCCGATGTTCTGAACG	824	(Ewers *et al.* 2007)
iucD	Aerobactin synthesis, Iron uptake chelat.	ACAAAAAGTTCTATCGCTTC CCTGATCCAGATGATGCT	714	(Janben *et al.* 2001)
irp2	Iron- repressible protein associated with Yersinia bactin synthesis	AAGGATTCGCTGTTACCGGA AACTCCTGATACAGGTGG	413	(Schubert *et al.* 1998)
iss	Increase serum survival	ATCACATAGGATTCTGCC CAGCGGAGTATAGATGCC	309	(Ewers *et al.* 2005)
kps (k1)	Capsule polysaccharide	TATAATTAGTAACCTGGGGC	927	(Knobl *et al.* 2012)

		GGCGCTATTGAATAAGACTG		
astA	Enteroaggregative heat-stable toxin	TGCCATCAACACAGTATATC TCAGGTCGCGAGTGACGG	116	(Franck *et al.* 1998)
cva/cvi	Structural genes of colicin V operon	TCCAAGCGGACCCCTTATAG CGCAGCATAGTTCCATGCT	598	(Ewers *et al.* 2007)

Table 2. Prevalence of virulence-associated genes in APEC field strains, as detected by PCR.

Samples(n)	***crl***	***fimH***	***csg***	***irp2***	***iss***	***iucD***	***cva***	***tsh***	***astA***	***K1***	***papC***
Turkey (100)	99	97	96	92	55	31	30	39	44	4	3
%	**99**	**97**	**96**	**92**	**55**	**31**	**30**	**39**	**44**	**4**	**3**
Chicken (35)	35	34	35	30	32	32	19	20	27	7	1
%	**100**	**97.1**	**100**	**85.7**	**91.4**	**91.4**	**54.2**	**57.1**	**77.1**	**20**	**2.8**
APEC (65)	65	63	60	55	61	60	52	38	11	20	5
%	**100**	**96.9**	**92.3**	**84.6**	**93.8**	**92.3**	**80**	**58.4**	**16.9**	**30.7**	**7.6**
Total (200)	199	194	191	177	148	123	101	97	82	31	9
%	**99.5**	**97**	**95.5**	**88.5**	**74**	**61.5**	**50.5**	**48.5**	**41**	**15.5**	**4.5**

Table 3. Percentages of *E. coli* isolates, susceptible, intermediate and resistant to antimicrobial agents.

Antimicrobial agent (µg)	n = 200		
	Susceptible	**Intermediate**	**Resistant**
Nalidixic acid (10µg)	122 (61%)	6 (3%)	72 (36%)
Amikacin (30µg)	184 (92%)	4 (2%)	13 (6.5%)
Ampicillin (10µg)	114 (57%)	7 (3.5%)	79 (39.5%)
Chloramphenicol (30µg)	184 (92%)	8 (4%)	8 (4%)
Colistin (10µg)	190 (95%)	4 (2%)	6 (3%)
Streptomycin (10µg)	165 (82.5%)	20 (10%)	15 (7%)
Trimethoprim (5µg)	174 (87%)	1 (0.5%)	25 (12.5%)

Figure Captions

Figure 1. Dendrogram of genomic similarity of 200 *E. coli* strains using ERIC-PCR result. C1-C31= *E. coli* clusters 1-31, S1-S25= *E. coli* singletons 1-25, T=turkey (green), Ch=chicken (orange) and A=APEC (blue).

Figure 2. Total detection rates of virulence-associated genes in isolates.

Figure 3. Percentage of carbon sources utilised in isolated *E. coli* strains from different sources grown on minimal medium with eleven different carbon source substrates.

Figure 4. Relationship between the phenotypic distance, and the genetic distance, resulting from comparisons between 200 *E. coli* strains, T=turkey (green), C=chicken (red) and A=APEC (blue). First component was genotypic characteristics and second component was phenotypic characteristics.

Figure 5. Correlation between the virulence genes, carbon sources utilised and antimicrobials agent between 200 *E. coli* strains. First component was genotypic characteristics and second component was phenotypic characteristics

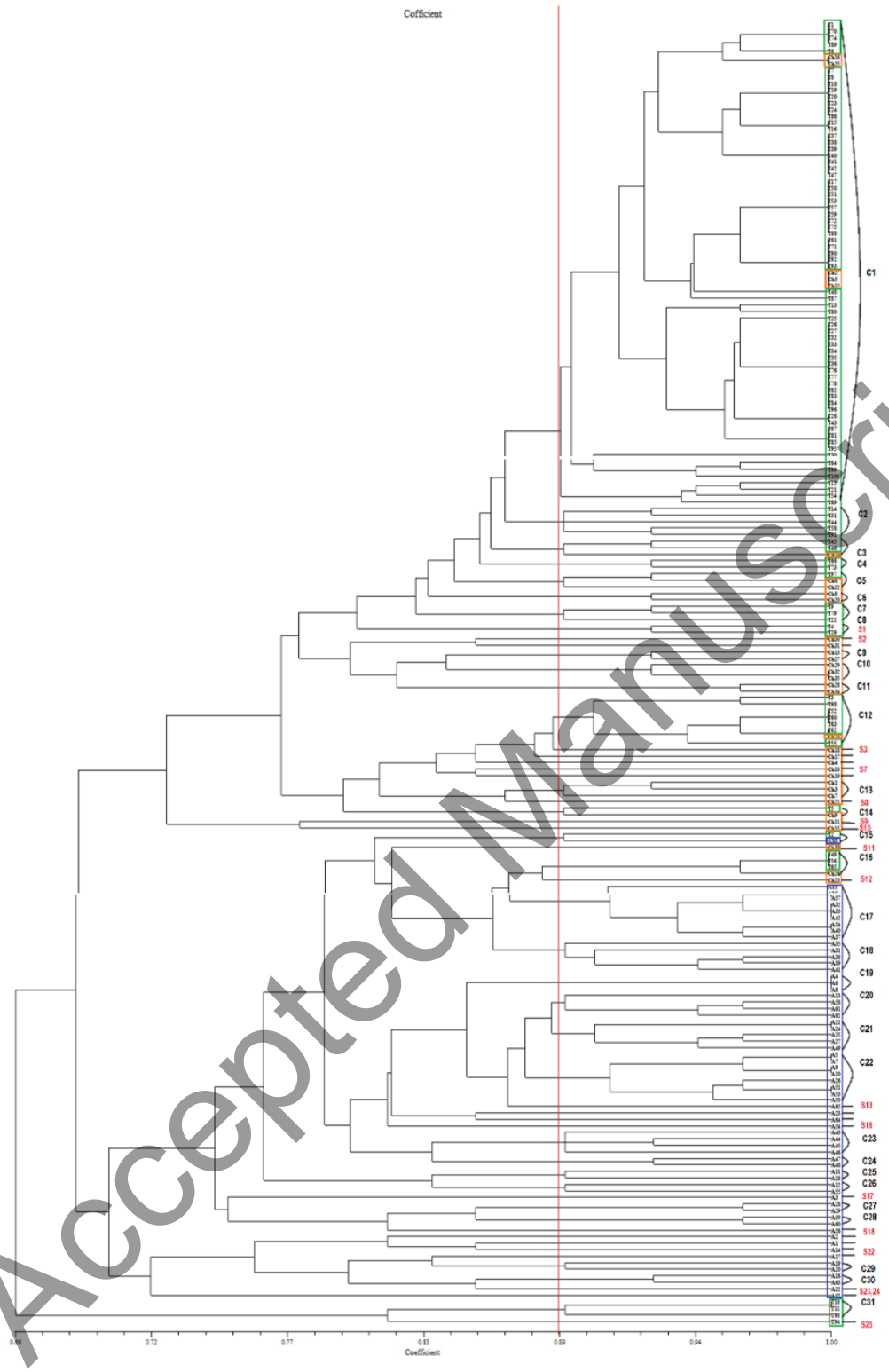

Figure 1

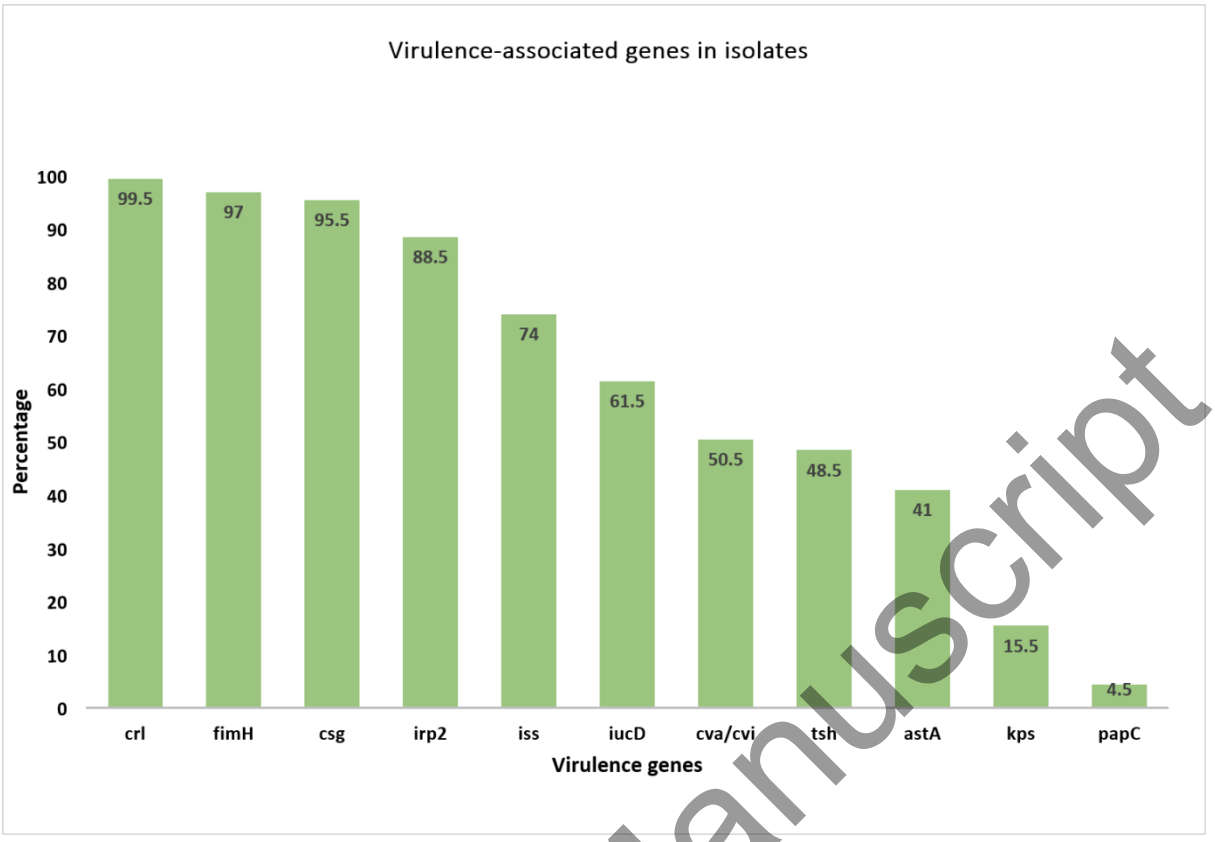

Figure 2

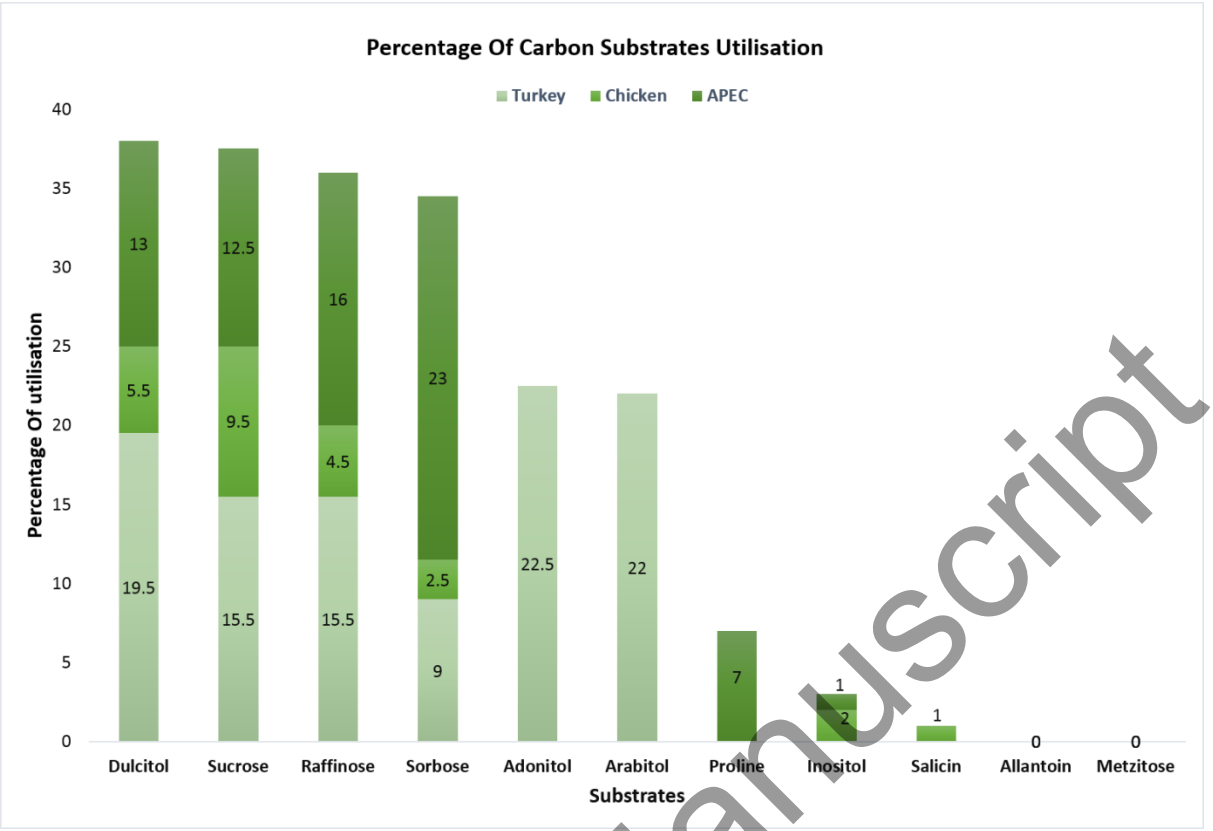

Figure 3

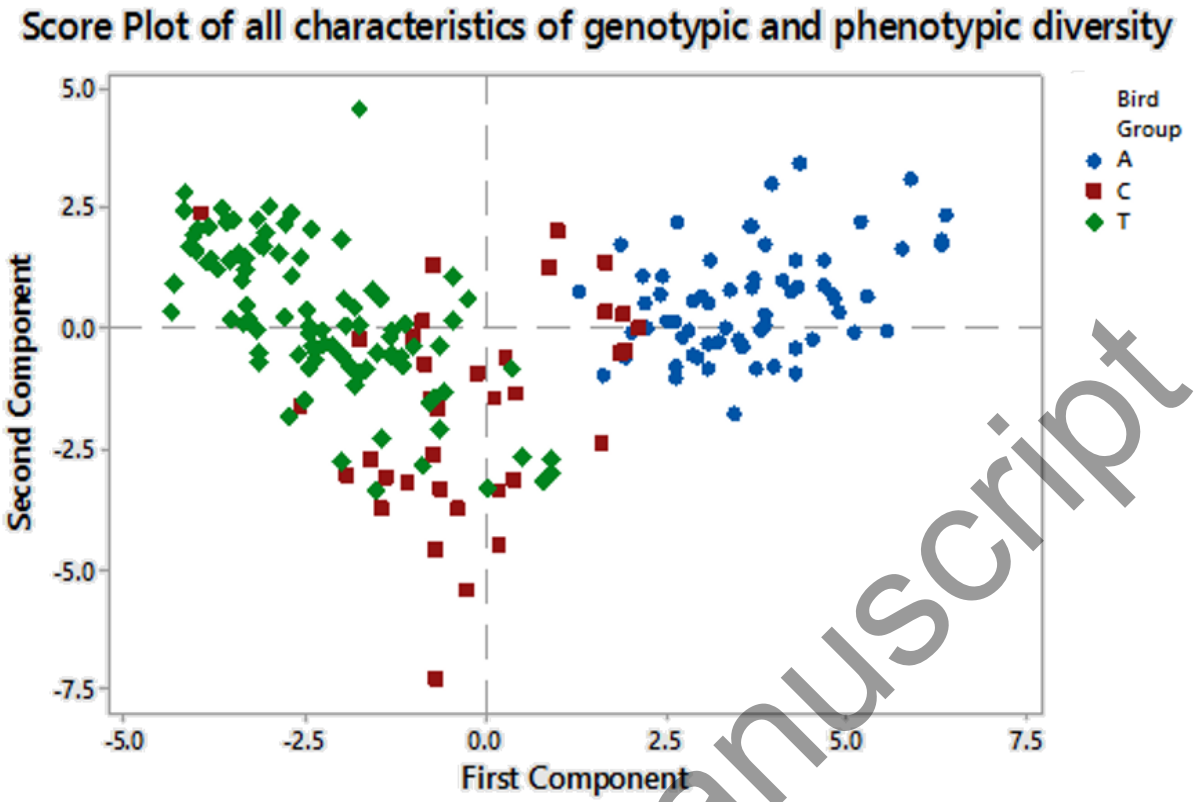

Figure 4

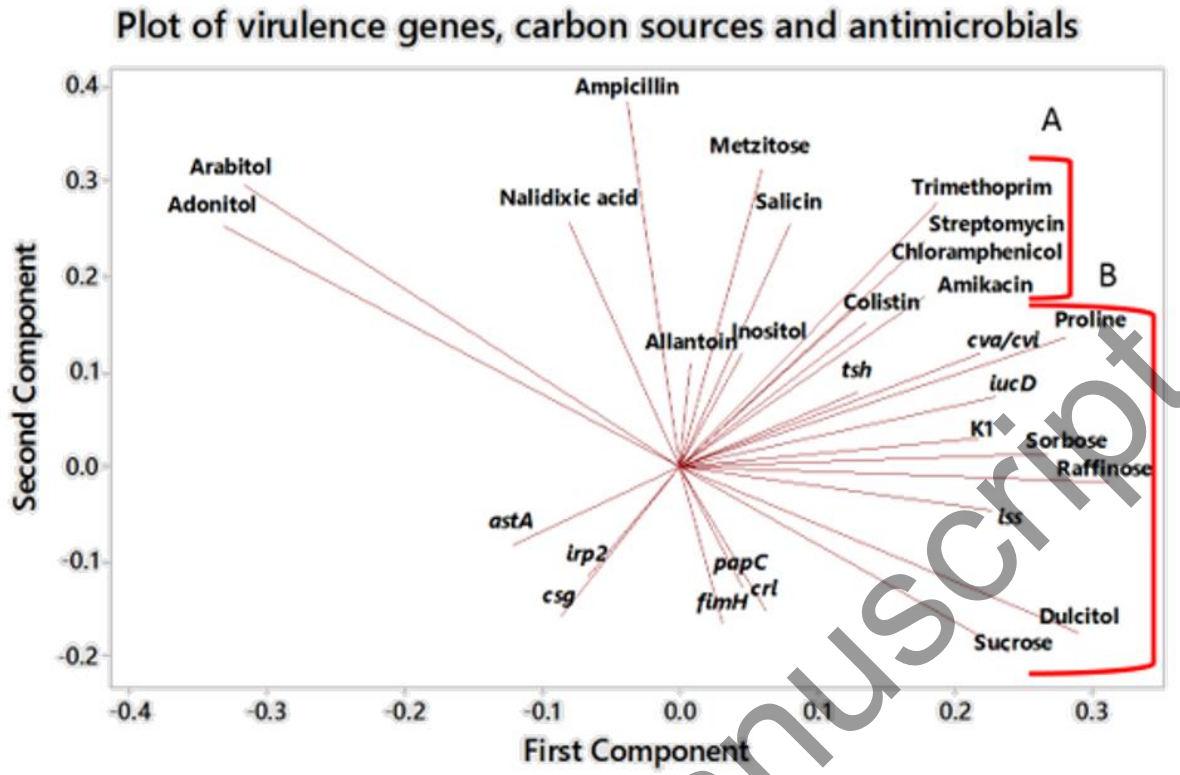

Figure 5